

SEQUENCE LISTING

<110> Penttila, Merja E.  
 Ward, Michael  
 Wang, Huaming  
 Valkonen, Mari J.  
 Saloheimo, Markku

<120> Increased Production of Secreted  
 Proteins by Recombinant Eukaryotic Cells

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<140> US 09/816,227  
 <141> 2001-03-23

<150> US 09/534,692  
 <151> 2000-03-24

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 <213> Trichoderma reesei

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Gln Asp Ala Glu Asp Asp Glu Ser His Ser Thr Ser Ala Thr Ala Pro						
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Ser Thr Ser Glu Lys Lys Pro Val Lys Lys Arg Lys Ser Trp Gly Gln						
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100	105	110				
Thr Glu Asp Glu Lys Glu Gln Arg Arg Val Glu Arg Val Leu Arg Asn						
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Gln Lys Thr Asn Leu Ile Leu Val Glu Glu Leu Asn Arg Phe Arg Arg						
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260	265	270				
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Val His Gln Asp Asp Gly Pro Phe Ser Ile Gly	His Ser Phe Gly Leu	
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Ser Ala Ala Leu Asp Ala Asp Arg Tyr Leu	Leu Glu Ser Gln Leu Leu	
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Ser Ala Ala Cys Phe Thr Asn Pro Leu Pro Ser	Asp Tyr Asp Phe Asp	
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Ile Asn Asp Phe Leu Thr Asp Asp Ala Asn His	Ala Ala Tyr Asp Ile	
385	390	395
Val Ala Ala Ser Asn Tyr Ala Ala Asp Arg	Glu Leu Asp Leu Glu	
405	410	415
Ile His Asp Pro Glu Asn Gln Ile Pro Ser Arg	His Ser Ile Gln Gln	
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<213> Aspergillus nidulans

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gatatgaaac	aacaaaacca	gttccttctt	cagcgtctcg	cccagatgga	ggctgagaac	660
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Glu Leu Pro Val Pro Lys Thr Asn Leu Pro Pro Arg Lys Arg Ala Lys  
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Arg Ala Ala Ala Gln Thr Ser Arg Glu Arg Lys Arg Leu Glu Met Glu  
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Pro Thr Pro Ser Val Thr Asp Tyr Ser Pro Thr Leu Lys Pro Ser Ser  
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<213> Trichoderma reesei

<400> 5

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Val Leu Pro Glu Pro Lys Thr Asn Leu Pro Pro Arg Lys Arg Ala Lys  
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180 185 190  
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Ala Ala Asn Pro Thr Val Asn Pro Ala Ser Leu Ser Pro Ser Leu Pro  
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Pro Ile Ser Asp Lys Glu Phe Gln Thr Lys Glu Glu Asp Glu Glu Gln  
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275 280 285  
Asp Ser Thr Gln Arg Pro Ala Val Ser Ile Gly Gly Asp Ala Ala Val  
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Ile Asn Asp Phe Leu Thr Asp Asp Ala Asn His Ala Ala Tyr Asp Ile  
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405 410 415  
Ile His Asp Pro Glu Asn Gln Ile Pro Ser Arg His Ser Ile Gln Gln  
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195 200 205  
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225 230 235 240  
Ser Ile Lys His Glu Pro Thr His Asp Leu Thr Ala Pro Leu Ser Asp  
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Pro Val Thr Leu Glu Asp Leu Glu Gln Thr Asn Gly Leu Ser Asp Ser  
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<212> DNA

<213> Aspergillus nidulans

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<212> PRT

<213> Aspergillus nidulans

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Trp	Asp	Cys	Gln	Ser	Ser	Gln	Ala	Val	Val	Glu	Phe	Val	Arg	Arg	Gly
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Ile	Ala	Ala	Lys	Gln	Asp	Leu	Tyr	Arg	Ile	Cys	Glu	Asn	Met	Met	Asp
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<212> DNA

<213> Trichoderma reesei

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<211> 438

<212> PRT

<213> Trichoderma reesei

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Tyr	Ala	Gln	Gly	Leu	Lys	Asp	Gly	Phe	Leu	Ala	Thr	Asp	Arg	Ala	Ile
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Arg Ala Ile Gly Asp Phe Glu Phe Lys Lys Ser Ala Glu Leu Ser Pro			
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Glu Asn Gln Ile Val Thr Ala Phe Pro Asp Val Glu Val His Glu Leu			
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Thr Glu Glu Asp Glu Phe Leu Val Ile Ala Cys Asp Gly Ile Trp Asp			
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Cys Gln Ser Ser Gln Ala Val Val Glu Phe Val Arg Arg Gly Ile Ala			
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Ala Lys Gln Asp Leu Asp Lys Ile Cys Glu Asn Met Met Asp Asn Cys			
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Leu Ala Ser Asn Ser Glu Thr Gly Gly Val Gly Cys Asp Asn Met Thr			
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Pro Glu Tyr Ala Glu Phe Arg Gly Pro Gly Val His His Asn Tyr Glu			
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Asp Ser Asp Ser Gly Tyr Asp Val Asp Ala Asp Ser Gly Gly Lys Phe			
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Glu Val Leu Thr Gly Ser Asp Asp Thr Glu Met Phe Asp Asn Ala Asp			
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Glu Asp Lys Asp Leu Ala Ser Gln Val Pro Lys Ser Ser Gly Lys Thr			
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Asp Ala Lys Glu Glu Thr Glu Ala Lys Pro Ala Pro Glu Ala Glu Ser			
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 <213> Aspergillus nidulans

<400> 11

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Asn	Val	Ile	Arg	Cys	Tyr	Cys	Arg	Glu	Gln	Ala	Lys	Gly	Phe	Phe	Tyr
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Pro	Asp	Ala	Phe	Pro	Gln	Leu	Val	Asn	Gly	Gly	Leu	Asp	Met	Pro	Asp
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Gly	Leu	Cys	Lys	Lys	Leu	Glu	Asp	Asn	Gln	Ser	Ser	Phe	Arg	Ala	Thr
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Glu	Ser	Ser	Glu	Pro	Ala	Val	Val	Asp	Pro	Gln	Thr	Asn	Arg	Arg	Ala
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 <213> Trichoderma reesei

<400> 13

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<211> 1232

<212> PRT

<213> Trichoderma reesei

<400> 14

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Ala Asp Asp Ala Asn Thr Arg Trp Tyr Ala Thr His Ala Ala Pro Asp  
50 55 60  
Val His Pro Glu Ala Lys Phe Asp Thr Val Asn Arg Lys Gln Lys Gln  
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Gln Ser Thr Ala Ser Pro Gln Gln His Gln Lys Tyr Arg Arg Ala Pro  
85 90 95  
Tyr Asp Tyr Ala Ser Lys Asp Lys Ala Gln Asn Arg Tyr Ala Gln His  
100 105 110  
Pro Ile Arg Glu Ser Glu Lys Pro Asn Tyr Val Lys Val Pro Asn Asp  
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His Thr Ser Arg His His Trp Pro Ser Ser Ala Ala Ser Gly Leu  
145 150 155 160  
Ala Ser Pro His Asn Ala Arg Ser Leu Glu Asp Trp Glu Val Glu Asp  
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Phe Val Leu Leu Ala Thr Val Asp Gly Asp Leu Tyr Ala Ser Asp Arg  
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Lys Thr Gly Arg His Leu Trp His Leu Glu Val Asp Gln Pro Val Val  
195 200 205  
Glu Thr Lys His Tyr Arg Thr Asn Asn Ser Val Leu Asp Asp Asp Tyr  
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225 230 235 240  
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Gly Phe Thr Met Lys His Leu Val Glu Glu Leu Ala Pro Tyr Ala Gly  
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275 280 285  
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Thr His Phe Leu Asn Pro Val Lys Ser Thr Gly Tyr His Gln Pro Pro		
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Ala Ser Asn Asn Ala His Ala Val Thr Asn Thr Val Pro Glu Glu Pro		
545	550	555
Thr Ile Ile Thr Lys Val Lys Ala Leu Pro Gln Ser Ala Ala Asn Ser		
565	570	575
Val Ile Asp Phe Val Ser Asn Pro Ile Leu Ile Ile Phe Leu Ile Gly		
580	585	590
Ser Leu Ile Tyr Asn Glu Lys Lys Leu Arg Arg Ser Tyr His Arg Phe		
595	600	605
Arg Thr His Gly Thr Ile Lys Asp Val Tyr Pro Phe Phe Val Ile Glu		
610	615	620
Ser Glu Ala Gly Asp Glu Ser Gly Asp Asp Lys Asp Gly Val Phe Pro		
625	630	635
Ser Ser Pro Ser Pro Arg Ser Gln Pro Gln Asp Gln Asn Ala Glu Asp		
645	650	655
His Leu Ser Arg His Lys Val Glu Arg Asn Ala Gly Asp Gln Asp Lys		
660	665	670
Val Lys Asp Asn Arg Ser Leu His Asp Val Ser Asp Thr Leu Glu Pro		
675	680	685
Ser Asn Lys Thr Val Glu Lys Thr Ala Asp Val Val Lys Gln Val Asp		
690	695	700
Val Ala Gly Pro Asp Ala Pro Ser Thr Asp Ser Asn Gly Ala Ala Pro		
705	710	715
Glu Lys Lys Lys Ala His Arg Gly Arg Arg Gly Gly Val Lys His		
725	730	735
Arg Lys Gly Arg Pro Thr Asp Gly Ser Gln Ser His Glu Asn Asp Pro		
740	745	750
Ala Leu Thr Thr Val Asp Glu Ala Val Ser Asn Ala Lys Lys Leu Gly		
755	760	765
Asp Arg Pro Ser Leu Glu Pro Asp Val Met Thr Ile Tyr Asn Asp Met		
770	775	780
Gln Ala Val Thr Gly Ser Val Ile Ser Met Gly Asn Ile Glu Val Asp		
785	790	795
Thr Asp Val Glu Leu Gly Met Gly Ser Asn Gly Thr Val Val Phe Ala		
805	810	815
Gly Arg Phe Asp Gly Arg Asp Val Ala Val Lys Arg Met Thr Ile Gln		
820	825	830
Phe Tyr Asp Ile Ala Thr Arg Glu Thr Lys Leu Leu Arg Glu Ser Asp		
835	840	845
Asp His Pro Asn Val Ile Arg Tyr Tyr Ser Gln Val Gln Arg Gly Asp		
850	855	860
Phe Leu Tyr Ile Ala Leu Glu Arg Cys Ala Ala Ser Leu Ala Asp Val		
865	870	875
		880

Ile Glu Lys Pro Tyr Ala Phe Gly Glu Leu Ala Lys Ala Gly Gln Lys  
                   885                  890                  895  
 Asp Leu Pro Gly Val Leu Tyr Gln Ile Thr Asn Gly Ile Ser His Leu  
                   900                  905                  910  
 His Ser Leu Arg Ile Val His Arg Asp Leu Lys Pro Gln Asn Ile Leu  
                   915                  920                  925  
 Val Asn Leu Asp Lys Asp Gly Arg Pro Arg Leu Leu Val Ser Asp Phe  
                   930                  935                  940  
 Gly Leu Cys Lys Lys Leu Glu Asp Arg Gln Ser Ser Phe Gly Ala Thr  
                   945                  950                  955                  960  
 Thr Gly Arg Ala Ala Gly Thr Ser Gly Trp Arg Ala Pro Glu Leu Leu  
                   965                  970                  975  
 Leu Asp Asp Asp Gly Gln Asn Pro Ala Ala Ile Asp Ser Ser Thr His  
                   980                  985                  990  
 Ser Gly Ser His Thr Ile Leu Val Gly Asp Pro Asn Ser Leu Ser Asn  
                   995                  1000                  1005  
 Gly Gly Arg Ala Thr Arg Ala Ile Asp Ile Phe Ser Leu Gly Leu Val  
                   1010                  1015                  1020  
 Phe Phe Tyr Val Leu Thr Asn Gly Ser His Pro Phe Asp Cys Gly Asp  
                   1025                  1030                  1035                  1040  
 Arg Tyr Met Arg Glu Val Asn Ile Arg Lys Gly Asn Tyr Asn Leu Asp  
                   1045                  1050                  1055  
 Pro Leu Asp Ala Leu Gly Asp Phe Ala Tyr Glu Ala Lys Asp Leu Ile  
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 Ala Ser Met Leu Gln Ala Ser Pro Lys Ala Arg Pro Asp Ser Arg Glu  
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 Val Met Ala His Pro Phe Phe Trp Ser Pro Lys Lys Arg Leu Ala Phe  
                   1090                  1095                  1100  
 Leu Cys Asp Val Ser Asp Ser Leu Glu Lys Glu Val Arg Asp Pro Pro  
                   1105                  1110                  1115                  1120  
 Pro Ala Leu Val Glu Leu Glu Arg His Ala Pro Glu Val Ile Lys Gly  
                   1125                  1130                  1135  
 Asp Phe Leu Lys Val Leu Thr Arg Asp Phe Val Glu Ser Leu Gly Lys  
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 Gln Arg Lys Tyr Thr Gly Asn Lys Leu Leu Asp Leu Leu Arg Ala Leu  
                   1155                  1160                  1165  
 Arg Asn Lys Arg Asn His Tyr Glu Asp Met Ser Asp Ser Leu Lys Arg  
                   1170                  1175                  1180  
 Ser Val Gly Ser Leu Pro Asp Gly Tyr Leu Ala Tyr Trp Thr Val Lys  
                   1185                  1190                  1195                  1200  
 Phe Pro Met Leu Leu Thr Cys Trp Asn Val Val Tyr Asn Leu Glu  
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 <212> DNA  
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 ttatgacacg gtggcctgtg ttcctgtgac acgggcaagc agacgtcctc cacaagctgt 120  
 gtcgacacctac atcaccgtcc tcccttgag tgcggttaag ataaggctca tagtaaatcg 180  
 attgatccac aattaaagat caatcacctg tcacgcttga aatgatggaa gaagcattct 240  
 ctccagtcga ctccctcgcc ggctccccga cgccctgagtt gccattgttg acagtgtccc 300  
 360

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agaaggctgt	gaagaagaga	aagtcatggg	gccaggaatt	gccagtcgg	aagactaact	480
tgcggccaaag	gaaacgggccc	aagactgaag	atgagaaaaga	gcaacgtcg	atcgagcgcg	540
ttcttcgcaa	tcgtggcga	gcacaaacat	cacggcgacg	caagaggctc	aaaatggaga	600
agttggaaaa	tgagaagatt	cagatggAAC	agcaaaacca	gttccttctg	caacgactat	660
cccagatgga	agctgagaac	aatcgcttaa	accaacaagt	cgctcaacta	tctgtgagg	720
tccggggctc	ccgtggcaac	actccaaAGC	ccggctcccc	cgtctcagct	tctccatccc	780
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ttcacccgtt	gggtcagatt	cttcagtcct	tgaagacggg	ttcgcccttg	acgttctcg	1140
cgaggagat	ctatcagcat	ttccatttga	ttctatgggt	gatttcgacc	ccgaatctgt	1200
tggcttcgaa	ggcatcgagc	cggggcacgg	tcttcggat	gagacttctc	gccagacttc	1260
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aatggagctg	ggagtctttg	ttgaccttgg	cgtggacgat	agacctactc	gaacagccgg	1440
gacgacgcaa	acgaatctt	agcggtttga	aatcagcgaa	aactgacgg	cgaagtaata	1500
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tgacgtctct	ccttatgggc	aagcatagtt	gaggttccgg	ctgtaaatta	tcataaatcc	1620
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   20   25                                   30  
 Ala Ser Pro Val Met Thr Arg Trp Pro Val Phe Leu Met Met Glu Glu  
   35   40                                   45  
 Ala Phe Ser Pro Val Asp Ser Leu Ala Gly Ser Pro Thr Pro Glu Leu  
   50   55                                   60  
 Pro Leu Leu Thr Val Ser Pro Ala Asp Thr Ser Leu Asp Asp Ser Ser  
   65   70                                   75                                   80  
 Val Gln Ala Gly Glu Thr Lys Ala Glu Glu Lys Lys Pro Val Lys Lys  
   85   90                                   95  
 Arg Lys Ser Trp Gly Gln Glu Leu Pro Val Pro Lys Thr Asn Leu Pro  
   100   105                                   110  
 Pro Arg Lys Arg Ala Lys Thr Glu Asp Glu Lys Glu Gln Arg Arg Ile  
   115   120                                   125  
 Glu Arg Val Leu Arg Asn Arg Ala Ala Ala Gln Thr Ser Arg Glu Arg  
   130   135                                   140  
 Lys Arg Leu Glu Met Glu Lys Leu Glu Asn Glu Lys Ile Gln Met Glu  
   145   150                                   155                                   160  
 Gln Gln Asn Gln Phe Leu Leu Gln Arg Leu Ser Gln Met Glu Ala Glu  
   165   170                                   175  
 Asn Asn Arg Leu Asn Gln Gln Val Ala Gln Leu Ser Ala Glu Val Arg  
   180   185                                   190  
 Gly Ser Arg Gly Asn Thr Pro Lys Pro Gly Ser Pro Val Ser Ala Ser  
   195   200                                   205  
 Pro Thr Leu Thr Pro Thr Leu Phe Lys Gln Glu Arg Asp Glu Ile Pro  
   210   215                                   220

Leu Glu Arg Ile Pro Phe Pro Thr Pro Ser Ile Thr Asp Tyr Ser Pro  
 225 230 235 240  
 Thr Leu Arg Pro Ser Thr Leu Ala Glu Ser Ser Asp Val Thr Gln His  
 245 250 255  
 Pro Ala Val Ser Val Ala Gly Leu Glu Gly Glu Gly Ser Ala Leu Ser  
 260 265 270  
 Leu Phe Asp Val Gly Ser Asn Pro Glu Pro His Ala Ala Asp Asp Leu  
 275 280 285  
 Ala Ala Pro Leu Ser Asp Asp Phe His Arg Leu Phe Asn Val Asp  
 290 295 300  
 Ser Pro Val Gly Ser Asp Ser Ser Val Leu Glu Asp Gly Phe Ala Phe  
 305 310 315 320  
 Asp Val Leu Asp Gly Gly Asp Leu Ser Ala Phe Pro Phe Asp Ser Met  
 325 330 335  
 Val Asp Phe Asp Pro Glu Ser Val Gly Phe Glu Gly Ile Glu Pro Pro  
 340 345 350  
 His Gly Leu Pro Asp Glu Thr Ser Arg Gln Thr Ser Ser Val Gln Pro  
 355 360 365  
 Ser Leu Gly Ala Ser Thr Ser Arg Cys Asp Gly Gln Gly Ile Ala Ala  
 370 375 380  
 Gly Cys  
 385

<210> 17  
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<210> 18  
 <211> 44  
 <212> PRT  
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<400> 18  
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 Ala Ser Pro Val Met Thr Arg Trp Pro Val Phe Leu  
 35 40

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 <211> 342  
 <212> PRT  
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 20 25 30  
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 35 40 45  
 Pro Val Lys Lys Arg Lys Ser Trp Gly Gln Glu Leu Pro Val Pro Lys

50	55	60
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Gln Arg Arg Ile Glu Arg Val	Leu Arg Asn Arg Ala Ala	Ala Gln Thr
85	90	95
Ser Arg Glu Arg Lys Arg	Leu Glu Met Glu Lys Leu	Glu Asn Glu Lys
100	105	110
Ile Gln Met Glu Gln Gln Asn	Gln Phe Leu Leu Gln Arg	Leu Ser Gln
115	120	125
Met Glu Ala Glu Asn Asn Arg	Leu Asn Gln Gln Val	Ala Gln Leu Ser
130	135	140
Ala Glu Val Arg Gly Ser Arg Gly Asn Thr	Pro Lys Pro Gly Ser Pro	
145	150	155
Val Ser Ala Ser Pro Thr Leu Thr Pro	Thr Leu Phe Lys Gln Glu Arg	
165	170	175
Asp Glu Ile Pro Leu Glu Arg Ile	Pro Phe Pro Thr Pro Ser	Ile Thr
180	185	190
Asp Tyr Ser Pro Thr Leu Arg Pro	Ser Thr Leu Ala Glu	Ser Ser Asp
195	200	205
Val Thr Gln His Pro Ala Val	Ser Val Ala Gly	Leu Glu Gly Glu Gly
210	215	220
Ser Ala Leu Ser Leu Phe Asp Val	Gly Ser Asn Pro Glu Pro His	Ala
225	230	235
Ala Asp Asp Leu Ala Ala Pro	Leu Ser Asp Asp Asp Phe His	Arg Leu
245	250	255
Phe Asn Val Asp Ser Pro Val	Gly Ser Asp Ser Ser Val	Leu Glu Asp
260	265	270
Gly Phe Ala Phe Asp Val Leu Asp	Gly Gly Asp Leu Ser Ala	Phe Pro
275	280	285
Phe Asp Ser Met Val Asp Phe Asp Pro	Glu Ser Val Gly Phe Glu Gly	
290	295	300
Ile Glu Pro Pro His Gly Leu Pro	Asp Glu Thr Ser Arg Gln	Thr Ser
305	310	315
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325	330	335
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340		

<210> 20  
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36

<210> 21  
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<212> DNA  
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<220>  
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<400> 21		
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ccggccgggtc ac	72	
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<211> 75		
<212> DNA		
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<210> 24		
<211> 21		
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caattgctcg ctcttacatt gaat	24	
<210> 26		
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<223> primer		

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gtaatacgac tcactatagg gc		22

<210> 32		
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<211> 24
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ccgcaacacg acacggcagg caac                                24

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<220>
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ctaggttagac gttgtatTTT g                                21

<210> 39
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tcgaacggat ccgaaaagaa gcccgtaag aagagg                36

<210> 40
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<210> 41
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<210> 42

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<210> 48
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<400> 48
caatttcaat acgggtggac 20

<210> 49
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<400> 49
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<210> 51
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<210> 52

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<400> 54		
caatatattt ctgaaccagt acg		23
<210> 55		
<211> 45		
<212> RNA		
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<400> 55		
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<210> 56		
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<211> 28		
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<213> Saccharomyces cerevisiae		
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<210> 58		

<211> 60  
 <212> DNA  
 <213> Trichoderma reesei

<400> 58  
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<210> 59  
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 <212> DNA  
 <213> Aspergillus nidulans

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 <212> PRT  
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 20 25 30  
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 35 40 45  
 Cys Ser Leu Leu Glu Asn Leu Leu Asn Ser Val Asn Leu Glu Lys Leu  
 50 55 60  
 Ala Asp His Glu  
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<210> 61  
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